REMARKS

Prior to this amendment claims 1-3 were pending and claims 4-31 were withdrawn from consideration by the Examiner. By this amendment claims 1 and 4-31 have been cancelled and new claims 32-45 added. Therefore, claims 2, 3 and 32-45 are currently pending. Certain typographical errors have been corrected, the SEQUENCE LISTING has been replaced with a more extensive listing and the new SEQ ID NOS have been incorporated in the specification.

The Invention

The inventors have discovered that the TSP-1 binding sequences of HRGP are useful in inhibiting the antiangiogenic activity of TSP-1. Specifically, the inventors have determined that proteins which include a thrombospondin-binding motif of HRGP are useful as pharmaceutical formulations for inhibiting the antiangiogenic activity of TSP-1 and thus stimulating angiogenesis. Such stimulation of angiogenesis is useful in treating various conditions and diseases, such as, for instance heart disease.

Objection to the specification

In the Office Action of July 16, 2002 the Examiner required that SEQ ID NO identifiers be inserted for amino acid and nucleotide sequences that appear in the specification.

The SEQ ID NOS have been inserted by amendment hereinabove as required.

Rejections of the claims

In the Office Action of July 16, 2002 the Examiner rejected claim 1 under 35 U.S.C. §101 as directed to non-statutory subject matter. Claim 1 has been cancelled herein. Therefore the rejection under 35 U.S.C. §101 is moot and must be withdrawn.

At page 3 of the Office Action claims 1-3 were rejected under 35 U.S.C. §112, first paragraph as allegedly containing subject matter that was not described in the specification so as to enable one of skill in the art to make or use the invention.

According to the Examiner, the specification discloses thrombospondin-binding motifs of SEQ ID NOS 1 and 2, but does [not] indicate what the additional sequences may encompass. Citing *Ex parte Forman* (230 USPQ 546, BPAI 1986), the Examiner stated that an undue burden of experimentation is required by one of ordinary skill in the art in order to make or use the invention as claimed.

Applicants respectfully disagree. The essential sequences and properties of the claimed proteins are fully described in the specification. SEQ ID NOS: 1-13 are provided as the essential thrombospondin-binding motifs of HRGP, one or more of which are present in the proteins of the pharmaceutical composition of the claimed invention.

The specification teaches several methods for assaying the claimed proteins, including: competition with CD36 (at page 47, last paragraph), and assaying the effect on the anti-angiogenic effect of TSP-1 *in vitro* (at page 48 second paragraph) and *in vivo* (page 48, third paragraph to page 49 last paragraph). It is well within the capacity of the artisan of

ordinary skill to determine the thrombospondin-binding activity of the proteins of the invention.

Therefore, Applicants respectfully traverse the rejection of claims 1-3 under 35 U.S.C. §112, first paragraph, which should be withdrawn.

At page 4, the Examiner rejected claims 1-3 under 35 U.S.C. §112, second paragraph as allegedly failing to provide a written description of the invention in full, clear, concise and exact terms or in sufficient detail that one of skill in the art can reasonably conclude that the applicant had possession of the invention at the time the application was filed.

According to the Examiner, the specification does not reasonably provide a written description of a protein comprising a thrombospondin-binding motif of HRGP. The Examiner then stated that the disclosure does not indicate that Applicant had possession of every protein comprising a thrombospondin-binding motif of HRGP with unknown sequences on one or both sides of the motif.

Further, the Examiner stated that there is no actual reduction to practice or sufficient descriptive information such as structural features, which are critical to protein activity, or a complete detailed description of the unknown sequence(s) adjacent the thrombospondin-binding motif of HRGP.

Applicants remind the Examiner that possession of every one of the claimed proteins comprising a thrombospondin-binding motif of HRGP with unknown sequences on one or both sides of the motif is not the correct standard for written description of the invention. The patent laws and regulations merely require that the invention be described in such a way as to convince one of ordinary skill that the inventor(s) had possession of the claimed

invention at the time the invention was filed. There is no requirement that the inventors elaborate each and every possible sequence of the claimed proteins.

The inventors have clearly demonstrated possession of the invention in the extensive description of the sequences that are required to be present in the proteins of the claimed pharmaceutical compositions of the invention both in the figures (Figure 1) and the text of the specification (at pages 10-12 and 13-15).

In view of the amendments and remarks submitted herein, reconsideration of the objections and rejections issued in the Office Action of July 16, 2002 is respectfully requested.

If any further issues remain to be addressed, the Examiner I respectfully invited to contact the undersigned attorney for Applicants at the telephone number listed below.

Respectfully submitted,

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EDITED VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

Please note, for the Examiner's convenience, additions are indicated in bold and deletions are indicated with a strikethrough.

On Page 3, please amend the first paragraph beginning with "Although TSP" to read as follows:

Although TSP-1 interacts with a number of distinct cellular receptors, CD36 has been recognized as the critical anti-angiogenesis receptor for TSP-1 (12, 13). The binding of TSP-1 to CD36 is mediated by the peptide sequence cysteine-serine-valine-threonine-cysteine-glycine (CSVTCG) **(SEQ ID NO. 14)**, the same type I repeat shown to have anti-angiogenic activity. (14, 15).

On Page 9, please amend the last paragraph beginning with "Figure 1" to read as follows:

Figure 1. HRGP contains CLESH-1 homology (thrombospondin-binding) motifs. Amino acid sequence alignment of CD36/LIMPII TSP binding motifs with homologous sequences in HRGP. Results of pattern-based search (BEAUTY) using CD36 exon 5 coding region (CD36 aa 95-143) **within SEQ ID NO: 18** as query identified a split CLESH-1 motif in HRGP (SEQ ID NO.1: aa 443-517). Optimization of alignments using SIM, ALIGN, and LALIGN programs also identified additional repeating motifs (SEQ ID NO.2: shown, aa ~~173-231~~ **173-230**). Amino acids identical between HRGP and either CD36 or LIMPII are highlighted white on black. Bold residues and pattern symbols represent conservative substitutions according to the following groups: basic [KRH, (+)]; acidic [DE, (-)]; charged [KRH, DE, ($)]; aromatic [YFW,(@)]; aliphatic [AG, (a)]; short chain [GA, STP, (!)]; hydrophobic [AGP, IVL, FM, (Δ)]; polar/hydrophilic [ST, KRH, DNEQ, CWY, (±)], hydroxyl [STY], and nonpolar/branched [IVL]. GenBank™/EMBL accession numbers: huHRGP, P04196; huCD36, M24795; huLIMPII, D12676.

On Page 10, please amend the last paragraph beginning with "The thrombospondin-binding" to read as follows:

The thrombospondin-binding motifs are disclosed as amino acid sequences 443-517 **(SEQ ID NO. 1)** and ~~173-231~~ **173-230 (SEQ ID NO. 2)** of HRGP as shown in Figure 1. The full amino acid sequence (1-525) of human HRGP is provided in GenBank under accession number P04196.

On Page 12, please amend the first paragraph beginning with "The thrombospondin-binding" to read as follows:

The thrombospondin-binding motifs of the present invention also include functional fragments and homologs of the amino acid sequences 443-517 **(SEQ ID NO. 1)** and ~~173-231~~ **173-230 (SEQ ID NO. 2)** of HRGP (see Figure 1) that retain the ability to bind TSP-1. The functional fragments may include combinations of sequences taken from the amino acid sequence 443-517 **(SEQ ID NO. 1)** and from the amino acid sequence ~~173-231~~ **173-230 (SEQ ID NO. 2)** of HRGP. Homologs of the amino acid sequences 443-517 **(SEQ ID NO. 1)** and ~~173-231~~ **173-230 (SEQ ID NO. 2)** of HRGP include sequence variants of each of these sequences, fragments of these variants and combinations of the fragments of these variants.

On Page 13, please amend the last paragraph beginning with "Functional fragments" to read as follows:

Functional fragments of thrombospondin-binding motifs are defined as those portions of the amino acid sequences shown in Fig. 1 which retain thrombospondin binding activity. Such thrombospondin-binding fragments include, for example, regions 443-517 (SEQ ID NO. 1) and ~~173-231~~ **173-230 (SEQ ID NO. 2)**; also smaller fragments such as 443-451 (SEQ ID NO. 3); 452-480 (SEQ ID NO. 4); 489-517 (SEQ ID NO. 5); and also 173-179 (SEQ ID NO. 6); 180-200 (SEQ ID NO. 7); and ~~201-231~~ **201-230** (SEQ ID NO. 8).

On Page 14, please amend the first full paragraph beginning with "The thrombospondin-binding sites" to read as follows:

The thrombospondin-binding sites may be comprised of any combination of the

above-named fragments. The number of fragments may be any number from 2-10, for example

443-480 (SEQ ID NO. 9) plus 452-480 (SEQ ID NO. 4);

452-480 (SEQ ID NO. 4) plus 489-517 (SEQ ID NO. 5);

443-451 (SEQ ID NO. 3) plus 489-517 (SEQ ID NO. 5);

also other fragments, for example 173-200 (SEQ ID NO. 10); and ~~180-231~~ **180-230** (SEQ ID NO. 11); may be used in combinations of sequences from the two regions, i.e. 443-517 (SEQ ID NO. 1) and ~~173-231~~ **173-230** (SEQ ID NO. 12) such as:

443-480 (SEQ ID NO. 13) plus ~~180-231~~ **180-230** (SEQ ID NO. 11);

443-480 (SEQ ID NO. 9) plus ~~201-231~~ **201-230** (SEQ ID NO. 8); and

~~180-231~~ **180-230** (SEQ ID NO. 11) plus 452-480 (SEQ ID NO. 4) and so on.

On Page 29, please amend the last paragraph beginning with "Optionally, the" to read as follows:

Optionally, the DNA that encodes the fusion protein is engineered so that the fusion protein contains a cleavable site between the protein and the fusion partner. Both chemical and enzymatic cleavable sites are known in the art. Suitable examples of sites that are cleavable enzymatically include sites that are specifically recognized and cleaved by collagenase (Keil et al., FEBS Letters 56:292-296 (1975)); enterokinase (Hopp et al., Biotechnology 6, 1204-1210 (1988) Prickett, K. S. et al., Biotechniques 7:580-589 (1989); LaVallie et al., J. Biol. Chem. 268:23311-23317 (1993)); factor Xa (Nagai et al., Methods Enzymol. 153:461-481 (1987)); and thrombin (Eaton et al., Biochemistry 25:505 (1986) and Chang, J. Y. Eur. J. Biocehem. 151:217-224 (1985)). Collagenase cleaves between proline and X in the sequence Pro-X-Gly-Pro wherein X is a neutral amino acid. Enterokinase cleaves after lysine in the sequence Asp-Asp-Asp-Asp-Lys **(SEQ ID NO. 15)**. Factor Xa cleaves after arginine in the sequence Ile-Glu or Asp-Gly-Arg. Thrombin cleaves between arginine and glycine in the sequence Arg-Gly-Ser-Pro **(SEQ ID NO: 19)**.

On Page 41, please amend the last paragraph beginning with "Reagents:" to read as follows:

Reagents: Recombinant human basic fibroblast growth factor (bFGF) was purchased from R & D Systems Inc. (Minneapolis, MN, USA) or from Research Diagnostics, Inc.(Flanders, NJ, USA). Rabbit antibody to HRGP was kindly supplied by Dr. Lawrence Leung, Stanford University, Palo Alto, CA. Murine monoclonal antibody to TSP-1 (11.4) has been previously described (16). Murine monoclonal antibody to CD36 (FA6) was obtained from the Vth International Workshop on Human Leukocyte Antigens (17). TSP-1 was purified from human platelet releasate by heparin affinity and anion exchange chromatography on Mono Q-Sepharose (Pharmacia Biotech Inc., Piscataway, NJ, USA) as described (3, 16). Radiolabeling was performed with $Na^{125}I$ (Amersham Life Science Inc., Arlington Heights, IL, USA) using immobilized chloramine T (IODO-BEADS, Pierce, Rockford, IL, USA) as described (18). Glutathione-S-transferase-CD36 fusion proteins (FP) have been previously described (19). HRGP was purified from human plasma by lys-plasminogen affinity chromatography as described (6). Purified proteins were incubated with polymyxin B-coated agarose (Sigma Chemical Co., St. Louis, MO, USA) to remove any potentially contaminating lipopolysaccharides (LPS) prior to use in cellular assays. Specific rabbit antibody to CSVTCG **(SEQ ID NO. 14)** was generated by subcutaneous immunization with KLH-coupled peptide. IgG was purified from serum by Protein A chromatography (Pierce).

On Page 44, please amend the first paragraph beginning with "*In vivo* subcutaneous" to read as follows:

In vivo subcutaneous Matrigel plug assays were performed as described (23). Briefly, 500 µl of Matrigel mixed with proteins or growth factors was injected subcutaneously near the abdominal midline of C57B1/6 mice. Gels were removed after 10 days, fixed in 1% paraformaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Immunohistochemistry was performed on unstained sections using an anti-human von Willebrand factor (vWF) IgG (Dako, Glostrup, Denmark) or isotype-matched control (Sigma) and a biotin-streptavidin-peroxidase antibody and development system (Vector Laboratories, Burlingame, CA) as described (24) and counterstained with Mayer's hematoxylin

(ImmunoGenex, San Ramon, CA, USA). After scanning, the degree of angiogenesis was determined by counts of vWF-positive blood vessels using Scion Image. For breast tissue, frozen sections of freshly obtained human breast carcinoma were incubated with rabbit antibody to HRGP or CSVTCG **(SEQ ID NO. 14)** or murine monoclonal antibody to TSP, or isotype-matched controls (Sigma), and developed as above. These studies were approved by the Institutional Animal Use Committee.

On Page 45, please amend the second paragraph beginning with "The binding of HRGP" to read as follows:

The binding of HRGP to TSP-1 was inhibited in the presence of 50 μM of the type-1 repeat synthetic peptide CSVTCG **(SEQ ID NO. 14)**, but not by a scrambled peptide, TVSGCC **(SEQ ID NO. 16)** or by an RGDS **(SEQ ID NO. 17)** peptide. However, the binding of plasminogen to TSP-1 was not inhibited by the synthetic peptides. Ligand blots show binding of radiolabelled HRGP to TSP-1 that had been subjected to SDS-PAGE and transferred to nitrocellulose, then developed by autoradiography.

On Page 45, please amend the third paragraph beginning with "The type 1 repeat" to read as follows:

The type 1 repeat of TSP-1 mediates binding to HRGP. HRGP binds to TSP-1 saturably, reversibly, and with high affinity (7nM) (6). Binding of HRGP to immobilized TSP-1 was inhibited by the TSP-1 hexapeptide CSVTCG **(SEQ ID NO. 14)**, whereas the control TSP-1 peptide (RGDS) **(SEQ ID NO. 17)** and the scrambled peptide (TVSGCC) **(SEQ ID NO. 16)** had no effect. As an additional control, the binding of plasminogen to TSP-1 was measured. Plasminogen binding was not inhibited by the CSVTCG **(SEQ ID NO. 14)** peptide (1B), further indicating that this interaction was not mediated by the type I repeats.

On Page 45, please amend the fourth paragraph beginning with "The TSP type 1 repeat" to read as follows:

The TSP type 1 repeat inhibits the binding of HRGP to TSP-1 in a concentration-dependent manner. Varying amounts of HRGP were added alone or in the presence of increasing concentrations of the peptide CSVTCG **(SEQ ID NO. 14)** to TSP-1 coated wells. Binding was measured by ELISA as described above. Inhibition of TSP-1 binding of HRGP by hexapeptide was concentration-dependent and reached maximum at 50 μM.

On Page 46, please amend the first paragraph beginning with "That the TSP-1-HRGP" to read as follows:

That the TSP-1-HRGP interaction is mediated by the TSP type I repeats is demonstrated by the following: Binding of radiolabelled HRGP to TSP-1 which had been resolved on SDS-PAGE and transferred to nitrocellulose was significantly decreased in the presence of anti-CSVTCG **(SEQ ID NO. 14)** antiserum and completely abolished by the CSVTCG **(SEQ ID NO. 14)** peptide (50μM). Inhibition by the TSP-1 type I repeat was concentration dependent and reached maximum at 50 μM.

On Page 46, please amend the third paragraph beginning with "TSP-1 and HRGP c0-localize in stroma of human" to read as follows:

TSP-1 and HRGP co-localize in stroma of human breast carcinoma. Frozen sections of freshly obtained tumor were incubated with monoclonal antibody to TSP-1, polyclonal anti-HRGP, or antiserum to CSVTCG **(SEQ ID NO. 14)**. Slides were developed with a peroxidase-conjugated avidin-biotin second antibody system and examined at 500X magnification. Panels taken from adjacent sections and showed stromal connective tissue bands staining with anti-TSP and anti-HRGP but not anti-CSVTCG **(SEQ ID NO. 14)**. Tumor cell stained with anti-TSP and anti-CSVTCG **(SEQ ID NO. 14)** but not with anti-HRGP.

On Page 47, please amend the first paragraph beginning with "In order to explore" to read as follows:

In order to explore whether the TSP type 1 repeat is involved in TSP-1-HRGP interactions in the breast cancer stroma, we developed a specific antibody to the CSVTCG **(SEQ ID NO. 14)** peptide. The antiserum was reactive to plasmodium falciparum circumsporozoite protein, known to contain the peptide, and to purified TSP-1, by Western blot. The ~~CSVCTG~~ **CSVTCG (SEQ ID NO. 14)** epitope was detectable intracellularly in the breast cancer cells where TSP-1 was detectable but HRGP was absent. However, in the tumor stroma where HRGP co-localized with TSP-1, there was no detectable ~~CSVCTG~~ **CSVTCG (SEQ ID NO. 14)** reactivity. This provides evidence that TSP-1 associates with HRGP *in vivo*, and that this interaction masks the type I epitope of TSP-1.

On Page 47, please amend the second paragraph beginning with "HRGP contains

CLESH-1" to read as follows:

HRGP contains CLESH-1 homology motifs. The binding site for TSP-1 on CD36 and other proteins is defined by homologous, evolutionarily conserved amino acid motifs known as CLESH-1 (18, 28, 29). As shown in figure 1, by sequence alignments of deduced amino acid sequences of HRGP we identified a region (aa 443-517) **(SEQ ID NO. 1)** with significant homology to the CLESH-1 domain of CD36 and the CD36-related protein LIMPII. (31% identity, 74% similarity). We also identified additional repeating motifs, including aa ~~173-231~~ **173-230 (SEQ ID NO. 2)** (20% identity, 70% similarity). Human immunodeficiency virus type I (HIV-1) also contains a CLESH-1 motif and may be susceptible to binding/immobilization by CLESH-1 binding and modulation of any biological activity dependent on the availability of the HIV-1gp120 CLESH-1 motif.

Please replace the Figures 1-3 as filed with the attached formal Figures 1-3.

IN THE CLAIMS

Please cancel claim 1 and withdrawn claims 4-31. Also, add new claims 32-45 as follows (All the pending claims 2, 3 and 32-45 are shown for the Examiner's convenience):

2. (Amended) A pharmaceutical composition comprising a protein which comprises a thrombospondin-binding motif of HRGP, in a pharmaceutically acceptable carrier, wherein the protein binds TSP-1 and thereby inhibits the antiangiogenic activity of TSP-1.

3. The pharmaceutical composition according to claim 2, wherein the composition is produced under GMP conditions or is of clinical grade, or both.

32. (New) The pharmaceutical composition according to claim 2, wherein the thrombospondin-binding motif of HRGP is a thrombospondin-binding motif of a mammalian HRGP.

33. (New) The pharmaceutical composition according to claim 32, wherein the mammalian HRGP is human HRGP.

34. (New) The pharmaceutical composition according to claim 2, wherein said protein is a mammalian HRGP.

35. (New) The pharmaceutical composition according to claim 34, wherein said mammalian HRGP is human HRGP.

36. (New) The pharmaceutical composition according to claim 2, wherein the thrombospondin-binding motif of HRGP is SEQ ID NO: 1.

37. (New) The pharmaceutical composition according to claim 2, wherein the thrombospondin-binding motif of HRGP is SEQ ID NO: 2.

38. (New) The pharmaceutical composition according to claim 2, wherein the protein comprises the amino acid sequences of both SEQ ID NO:1 and SEQ ID NO: 2.

39. (New) The pharmaceutical composition according to claim 2, wherein the protein comprises one or more of the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:13.

40. (New) The pharmaceutical composition according to claim39, wherein the protein comprises SEQ ID NO:9 and SEQ ID NO:4.

41. (New) The pharmaceutical composition according to claim 39, wherein the protein comprises SEQ ID NO:4 and SEQ ID NO:5.

42. (New) The pharmaceutical composition according to claim 39, wherein the protein comprises SEQ ID NO:3 and SEQ ID NO:5.

43. (New) The pharmaceutical composition according to claim 39, wherein the protein comprises SEQ ID NO:13 and SEQ ID NO:11.

44. (New) The pharmaceutical composition according to claim 39, wherein the protein comprises SEQ ID NO:9 and SEQ ID NO:8.

45. (New) The pharmaceutical composition according to claim 39, wherein the protein comprises SEQ ID NO:11 and SEQ ID NO:4.